GENETIC POLYMORPHISM IN GASTROPODS: A COMPARISON OF METHODS AND HABITAT SCALES

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ABSTRACT

We compare genetic differentiation in gastropods at two habitat scales, using two methodologies. For the pond pulmonate, *Lymnaea elodes* (Say), we present data on the degree of genetic variance for life histories by comparing variation in traits among full sib groups reared in a common field environment, for two source populations (one vernal, one permanent pond). For the same two populations, as well as a third in another vernal pond, we also present data on allozyme polymorphism. Finally, we contrast genic polymorphism occurring over a much broader habitat scale, using published literature on allozyme polymorphism found respectively in terrestrial, freshwater, and marine environments, and for snails having selfing, outcrossing, or parthenogenetic mating systems.

We found more genetic variation for life history traits in *Lymnaea elodes* occurring in a vernal pond, as variation among sib groups was significant for 5 out of 6 traits measured, versus only 2 out of 6 in a population from a permanent pond. We thus found that unpredictable habitats can favor greater levels of genetic variation in life histories. In contrast, genic polymorphism was similar in all three ponds, with from 27 to 33% of loci polymorphic, and mean heterozygosity ranging only from 8 to 10%. Genetic similarity was high for the two vernal ponds and lower for the more distant permanent pond. Divergence in heterozygosity did occur across broader habitat categories, with lower mean heterozygosity for snails in terrestrial habitats, and self-fertilizers in particular possessing significantly lower heterozygosity within this habitat. The literature survey also indicated more work on allozyme variation is needed in particular for freshwater pulmonates, and we suggest such work along with further work on variation in polygenic characters like life histories.

Although many studies have looked at variation in bioenergetics, life histories, and shell structure among populations of freshwater snails (see reviews in Russell-Hunter, 1978; Russell-Hunter and Buckley, 1983; and McMahon, 1983), little is known of the genetic basis of variation among or within populations for these traits (Brown, 1983). In contrast, quite a bit is known, from studies of allozyme variation, about levels of genic polymorphism in freshwater as well as marine snails (see reviews in Clarke *et al.*, 1978; Berger, 1983; Nevo *et al.*, 1983; Selander and Ochman, 1983). No studies have as yet attempted to study both the genetic basis for variation in life histories and genic polymorphism among and within the same populations of a species. We present such data on variation among sib groups of snails for a number of life history patterns, contrasting them among two populations of the pond snail *Lymnaea elodes* (Say). One population is from a vernal, the other a permanent pond in northern Indiana. For these same two populations, as well as a second vernal pond, we also present data on allozyme variation. To determine if trends in mean heterozygosity appear at a broader habitat scale than between populations, we also review the available literature on allozyme variation in freshwater, marine, and terrestrial gastropods. Within each of these broad habitat categories, we further divide populations as to their breeding systems, including selfing, outcrossing, and parthenogenetic reproduction to determine whether these reproductive modes have any broad effect on polymorphism.

For *Lymnaea elodes*, several studies have concentrated on proximal factors affecting population dynamics, including density dependence (Eisenberg, 1970), habitat productivity and permanence (Hunter, 1975; Brown *et al.*, 1985), and water temperature (Brown, 1979). Brown (1985) used transfer experiments to show that most variation among populations in life histories was due to habitat productivity.

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However, lack of divergence among populations could also be due to pronounced phenotypic and/or genetic variation within populations. For this reason we decided, using snails from both populations, to rear offspring from different sib groups in the same field environment to determine the scale of differences in life histories occurring across sib groups.

We decided to study allozyme polymorphism in the same populations of *Lymnaea elodes* for two reasons. First, genic polymorphism is probably an independent estimator of genetic variation in populations, when compared to genetic variation in phenotypes such as life histories (Lewontin, 1984). Second, little is known of allozyme variation among and within populations of lymnaeid snails or, for that matter, most other freshwater pulmonates. In contrast, allozyme variation has been studied in detail within and among populations of freshwater prosobranchs (Chambers, 1978, 1980; Selander *et al.*, 1978; Dillon and Davis, 1980; Karlin *et al.*, 1980; Selander and Ochman, 1983; Dillon, 1984). Marine gastropods, which are almost exclusively prosobranchs, have also been studied extensively for allozyme polymorphism. Studies have included many species of *Cerithium* (Ritte and Pashton, 1982; Lavie and Nevo, 1986), *Crepidula* (Hoagland, 1985; Woodruff *et al.*, 1986), *Littorina* (Ward and Warwick, 1980), marsh snails such as *Nassarius (Gooch* et al., 1972), thaidids (Garton, 1984; Garton and Stickle, 1985), and a deep-water species, *Bathybembix bairdii* (Dall) (Siebenaller, 1978).

The genetic structure of terrestrial gastropods is perhaps the best known, with studies ranging from slugs (Foltz *et al.*, 1982a, b, 1984) to a large number on shelled species such as *Cerion, Cepaea,* and *Partula* (see references listed in appendix). We have done the first survey to look explicitly for differences in polymorphism among gastropods across broad habitat categories, although several reviews have considered the effect of mating systems on heterozygosity (Selander and Kaufman, 1975; Nevo *et al.*, 1983; Selander and Ochman, 1983).

METHODS

THE SPECIES AND HABITATS

Lymnaea elodes is a common algivore in temporary ponds and marshes in the northern tier of states in the United States as well as Canada (Brown, 1979). Its life cycle length, fecundity, and shell growth are determined for the most part by habitat productivity and adult snail density (Eisenberg, 1970; Hunter, 1975; Brown, 1985). Adults reproduce in late spring and early summer, and juveniles and some adults estivate over late summer (if the pond dries) and winter (Brown *et al.*, 1985). *Lymnaea elodes* can be eliminated from more permanent habitats (e.g. lakes) by fish predators (Brown and DeVries, 1985). Snails for this study were collected from a vernal pond (Pond A, Brown, 1982) and a more permanent pond (Pond F, Brown, 1982) for experiments assessing variation among sib groups. The vernal pond usually dries by early July and the more permanent pond has water at least until August, after oviposition has been completed. Snails were also collected from a second vernal pond (Pond B, Brown, 1982) for the electrophoretic analyses. This pond usually dries in late July. The three ponds also differ in food levels, with the permanent pond having the greatest periphyton productivity (Brown *et al.*, 1985). All ponds are located in Noble County, Indiana within 30 km of Crooked Lake Biological Station, 33 km NW of Fort Wayne.

VARIATION AMONG SIB GROUPS

The permanent pond was selected as a common rearing site for both populations since higher food levels would not limit egg production (Brown *et al.*, 1985) and snails would be able to complete their life cycles before pond drying. Thirty juveniles were collected from the temporary pond and twenty-five juveniles from the permanent pond in early spring 1981. These snails were placed singly in flow-through containers in Pond F and reared through their entire life cycle. They were paired with a snail from the same source pond for a two week interval when they were between 12 mm and 14 mm shell length to allow outcrossing (lymnaeid snails, like other pulmonates, are hermaphroditic). Companion snails were removed before experimental snails reached 16 mm, the smallest recorded shell length at maturity in these populations (Brown *et al.*, 1985), so that egg laying would not be confounded between the two individuals. Since *Lymnaea elodes* outcrosses preferentially (Brown, 1979), we have assumed snails did not self-fertilize. At weekly intervals, we measured snails and removed all egg cases. Eggs and hatched juveniles were kept over winter in 6/ aquaria at 13°C in the laboratory to retard growth and maturation. In spring 1982, we placed an average of 3.5 full sib offspring per temporary pond parent, and 3.0 full sib offspring for each permanent pond parent back in the permanent pond. The same rearing methods were used for offspring as for their parents in the preceding season. A more detailed account of the rearing methods is given in Brown *et al.* (1985).

Six life history traits were measured for each offspring: (1) shell length at maturity; (2) relative age at maturity (days since start of experiment); (3) clutch size (average number of eggs per mass); (4) total fecundity; (5) shell length at death; (6) relative age at death (days since start of the experiment). Exact ages at maturity and death were impossible to determine, as offspring were not separated in the laboratory by date laid. The impact of differing growth rates of offspring over winter in the laboratory was minimized by using initial shell length as a covariate in an analysis of covariance (ANCOVA) that assessed differences among sib groups in each of the life history patterns.

ALLOZYME VARIATION

Approximately 150 snails were collected from each pond and were frozen at −60°C until analysis when the foot was ground in a chilled cell mill with 0.5 ml of deionized water. Supernatant was absorbed onto wicks of Whatman #3 filter paper and inserted into horizontal starch gels and subjected to electrophoresis for 3-5 hours at 35-55 mA and 200 volts. Gels were stained for the following enzyme systems: (1) acid phosphatase (ACP); (2) esterases (EST); (3) aspartate amino-

transferase (AAT); (4) glucose phosphate isomerase (GPI); (5) hexanol dehydrogenase (HEX); (6) leucine aminopeptidase (LAP); (7) malate dehydrogenase (MDH); (8) mannose-6-phosphate isomerase (MPI); (9) 6-phosphogluconate dehydrogenase (PGD); (10) sorbitol dehydrogenase (SDH); (11) superoxide dismutase (SOD). All loci were examined for all populations. Formulas for gel and electrode buffers, as well as stains for these enzyme systems were taken from Shaw and Prasad (1970), Selander *et al.* (1971), Chambers (1980), and Dillon and Davis (1980). Enzymes with multiple loci were numbered by mobility (1=fastest).

Allelic and genotypic frequences were calculated using the BIOSYS-1 FORTRAN program (Swofford and Selander, 1981) which also calculated the percentage of loci polymorphic (using the criterion that a second allele must have a frequency $\geq 5\%$). Mean observed and expected heterozygosities (over all loci) for each population and genetic distance indices between populations were also calculated. BIOSYS-1 uses the methods of both Rogers and Nei to calculate genetic distance.

In the literature survey, we either used reported heterozygosities, or calculated heterozygosity over all loci (including monomorphic loci as 0% heterozygous) if raw data on allelic or genotypic frequencies were reported. Each population was then catalogued by habitat and reported mating system. If we could not determine from the paper whether the gastropod was a selfer, outcrosser, or parthenogen, it was placed in the facultative selfing category along with species reported as having a mixed breeding strategy. Data were arc-sine transformed and subjected to ANOVA. The ideal design would be factorial, allowing us to look at the interactive effects of habitats and breeding systems. Due to empty cells, we were constrained, however, to perform two separate one way analyses. First we performed a oneway ANOVA over habitat categories. Second, we performed a oneway ANOVA over breeding systems within each habitat category. Duncan's *a posteriori* multiple range tests were used to compare means (at the 0.05 significance level) if the F statistic was significant.

RESULTS

VARIATION AMONG SIB GROUPS

For sib groups from the permanent pond, the covariate (initial shell length) had significant effects on four of the six life history traits (Table 1). In contrast, only two life history traits, shell length at maturity and clutch size, showed significant variation among sib groups. Variation among sib groups in life history traits was much more obvious in the temporary pond, with significant effects occurring for five of the six traits (Table 2). Initial shell length, however, still had significant effects on the same five traits. Thus, the ANCOVA suggests greater levels of genetic variation for life histories in the vernal pond population, when snails are reared in a common field environment. However, the inital size of individuals when introduced to containers also has substantial effects on life history variation.

Table 1. Analysis of Covariance, with initial shell length as the covariate, of six life history traits among sib groups from a permanent pond. Values are F statistics. One asterik indicates significance at the 0.05 level, two at the 0.01 level.

	SOURCES OF VARIATION			
Traits	Treatments	Covariate	Among Sib Groups	Within Sib Groups
Degrees Freedom	24	1	23	60
Age at Maturity	1.3	6.6*	<1	
Shell length at Maturity	2.9**	28.3**	1.8*	
Clutch Size	3.0**	6.8*	2.9**	
Total Fecundity	1.3	3.1	1.2	
Age at Death	1.7*	6.2*	1.5	
Shell Length at Death	<1	<1	<1	

Table 2. Analysis of Covariance, with initial shell length as the covariate, for six life history traits among sib groups from a temporary pond. Values are F statistics. One asterisk indicates significance at the 0.05 level, two at the 0.01 level.

	SOURCES OF VARIATION			
Traits	Treatments	Covariate	Among Sib Groups	Within Sib Groups
Degrees Freedom	30	1	29	81
Age at Maturity	6.2**	60.7**	4.3**	
Shell length at Maturity	3.6**	43.7**	2.3**	
Clutch Size	3.1**	9.2**	2.9**	
Total Fecundity	2.8**	24.2**	2.1**	
Age at Death	<1	<1	<1	
Shell Length at Death	2.4**	14.0**	2.0**	

ALLOZYME POLYMORPHISM

Patterns in allozyme polymorphisms were consistent across ponds. In all three populations, nine loci were monomorphic: ACP; EST-1; EST-4; GPI; HEX; MPI; PGD; SDH; SOD. In the snails from the first temporary pond (A), 26.7% of the loci were polymorphic, including EST-3, AAT, LAP-1 and MDH. In the second temporary pond, the LAP-2 locus was also polymorphic, with one-third of the loci polymorphic overall (Table 3). In the permanent pond, 26.7% of the loci were polymorphic, namely EST-2, EST-3, LAP-1, and MDH (Table 3).

Mean observed heterozygosity was also similar in each

Table 3. Allelic frequencies, mean expected and observed heterozygosities, and proportion of total loci polymorphic for three pond populations of *Lymnaea elodes*. N refers to number of individuals.

Locus	Allele	Temporary Pond	Second Temporary Pond	Permanent Pond
EST-2	A	0.99	1.0	0.32
	B	0.01	0.0	0.68
EST-3	A	0.07	0.01	0.19
	B	0.03	0.32	0.39
	C	0.90	0.67	0.42
AAT	A	0.85	0.63	1.0
	B	0.15	0.37	0.0
LAP-1	A	0.55	0.01	0.02
	B	0.32	0.35	0.29
	C	0.13	0.64	0.69
LAP-2	A	0.99	0.95	0.99
	B	0.01	0.05	0.01
MDH	A	0.72	0.56	0.94
	B	0.28	0.44	0.06
Mean Observed Heterozygosity (SE)		0.08(0.04)	0.10(0.04)	0.09(0.04)
Mean Expected Heterozygosity (SE)		0.10(0.05)	0.13(0.06)	0.11(0.06)
Percent of Loci Polymorphic		26.7	33.3	26.7
N		150	150	150

Table 4. Pairwise estimates of genetic distance among populations of the pond snail *Lymnaea elodes*. Nei's "unbiased" indices are above the main diagonal, Rogers' indices below.

	Temporary Pond	Second Temporary Pond	Permanent Pond
Temporary Pond	0.0	0.03	0.07
Second Temporary Pond	0.08	0.0	0.06
Permanent Pond	0.14	0.12	0.0

of the populations and ranged from 8 to 10% (Table 3). Mean expected heterozygosities calculated by BIOSYS-1 ranged from 10 to 13%, indicating some degree of heterozygote deficiency in all three ponds as is common in most molluscs. All pair-wise comparisons of the three populations show levels of genetic distance near 0.05 (Table 4), a level characteristic of populations within the same species (Avise, 1976). Both distance indices indicated snails from the permanent pond to be more dissimilar from each of the 2 temporary ponds than the 2 temporary ponds were from each other. This could be due to lower levels of gene flow, since the permanent pond is over 40 km from either of the temporary ponds, which are separated by only 2 km.

Although percent of polymorphic loci and mean observed heterozygosities were similar, there were some interesting differences in allele frequencies among the 3 populations (Table 3). The 2 temporary ponds had similar allele frequencies at EST-2 with allele A being most common. However, in the permanent pond population allele B predominated with a frequency of 0.68. For EST-3, allele C was most common in the temporary pond A population, but dropped to 67% in the second temporary pond population. In the permanent pond, allele A was more common than in ponds A or B. AAT had similar allelic frequencies in both temporary pond populations with allele A declining from 0.85 to 0.63 in the second temporary pond. The pond F population was monomorphic at this locus. Ponds B and F were similar in allelic frequencies at LAP-1 locus with allele C predominating. In contrast, in the pond A population allele A was most common. LAP-2 did not differ much in allelic frequencies among the 3 populations, although with the $\geq 5\%$ criterion LAP-2 was polymorphic only in the pond B population. MDH differed somewhat in allelic frequencies among the 3 populations although allele A was always more common.

LITERATURE SURVEY

Genic polymorphism has been much more extensively studied in terrestrial gastropods, with over twice as many populations represented than either of the other two habitat categories (Table 5). Outcrossing appeared the most common mating system in each habitat, and populations with obligate selfing were found only in terrestrial snails. Parthenogenetic populations have been studied only in freshwater snails (Table 5). The actual populations and heterozygosities used in the analysis are given in the appendix.

Table 5. Overall mean heterozygosity for habitats and mating systems. Means are weighted for sample size. Numbers in parentheses are sample size.

Mating System	Terrestrial	Freshwater	Marine
Selfers	0.0 (6)	—	—
Outcrossers	0.089 (34)	0.106 (14)	0.173 (13)
Parthenogens	—	0.207 (6)	—
Facultative Selfers	0.047 (10)	0.088 (1)	0.090 (2)
Overall mean	0.061 (50)	0.131 (21)	0.161 (15)

Mean observed heterozygosity was highly significantly different among habitat types ($F_{2,83} = 7.8$; $p < 0.001$). The *a posteriori* test revealed that only terrestrial populations had significantly lower average heterozygosity. Although average heterozygosity can be lowest in terrestrial snails simply because they alone possess selfing populations with no genic polymorphism (Table 5), there appears also to be a general trend, as terrestrial snails in both the outcrossing and

facultative selfing categories had the lowest observed heterozygosity of the three habitats. Within terrestrial gastropods, there was, as might be expected, a highly significant difference in mean heterozygosity among mating systems ($F_{2,47}$ = 16.6, $P < 0.0001$), and Duncan's multiple range test indicated selfers had a significantly lower average heterozygosity. As also might be expected, outcrossing gastropods had the highest average heterozygosity, and partial selfers had intermediate heterozygosities. In both freshwater and marine habitats there were no significant differences among mating systems in average heterozygosity ($P > 0.05$). Finally, this study of polymorphism in *Lymnaea elodes* reveals levels of heterozygosity just below the average for outcrossing freshwater snails as a group (Table 5), indicating the most probable mating system in these pond populations is mixed.

DISCUSSION

The results of the full sib analyses indicate greater levels of genetic variation for life history traits in snails drawn from a vernal pond population. Perhaps the more unpredictable nature of this habitat has favored the maintenance of genetic variation in life history traits. For example, the vernal pond has extremely unpredictable drying dates from year to year (Brown *et al.,* 1985). In wet years, juvenile recruitment is good, and adult densities are high enough the next year to depress fecundity by density dependence. In years with little rainfall, the vernal pond dries so early that juvenile and adult mortality are intense (Brown *et al.,* 1985). If genetic variation in life histories provides a range of age at reproduction, etc., then at least some individuals would successfully reproduce regardless of the drying date, and genetic variance for life history traits would be maintained. Interestingly, populations of pill clams in vernal ponds in Ohio also have more genetic variation than populations in permanent ponds (McCleod *et al.,* 1981; Burky, 1983). In addition, initial size of individuals introduced to containers also affected life history variation; as initial size increases so does clutch size, age at maturity, shell length at maturity, and age at death (Brown *et al.,* 1985).

In contrast, the electrophoretic data indicate little difference in polymorphism between any of the populations of *Lymnaea elodes.* Levels of polymorphism are very similar in both of the vernal ponds, and essentially the same set of loci vary in the permanent pond as well. Thus, interpretations on levels of genetic variation within and among populations based on the electrophoretic data do not agree with those based on variation among full sibs in life history traits. However, as Lewontin (1984) points out, when there is no known functional relationship between the allozymes and quantitative traits chosen (as in this case), there is no reason to expect a pattern to emerge when comparing the two between populations. Even if a functional relationship exists between the allozymes and quantitative traits, the relative lack of statistical power associated with gene frequency analyses would require a prohibitively large sample size to detect differences at the same level of statistical significance as the quantitative traits. The allozyme polymorphism data do indicate, however, little genetic differentiation among populations, similar to earlier transplant studies (Brown, 1985) suggesting little genetic divergence among populations in life histories. Compared to the average for all populations reported in the literature, mean heterozygosity in these populations of *L. elodes* is very near the value for outcrossing terrestrial pulmonates, slightly less than the average for outcrossing freshwater snails (again mostly dioecious prosobranchs) and much less than dioecious prosobranch marine snails. Therefore, these populations of *L. elodes* probably have a mixed breeding system, with some inbreeding occurring, if for no other reason than the fact that populations go through bottlenecks when ponds dry early (Brown *et al.,* 1985). Also, previous studies of mollusc populations indicate GPI, MPI, PGD, SOD, and HEX are virtually always polymorphic (Clarke *et al.,* 1978; Selander and Ochman, 1983). Interestingly, these loci were monomorphic in these 3 populations of *L. elodes*, possibly due to the recurrent bottlenecks.

However, the literature survey indicated there were differences in heterozygosity over broader habitat categories than these pond populations of *Lymnaea elodes.* Terrestrial pulmonates, regardless of the mating system, have the lowest heterozygosities. This could be due to the nature of their habitats. Terrestrial micro-environments hospitable to snails (the proper temperature and humidity, etc.) might be expected to be more patchily distributed than those in aquatic or marine habitats (Russell-Hunter, 1983). Furthermore, terrestrial snails are relatively immobile and might self-fertilize more than most have considered. Effective population sizes could therefore be low and inbreeding might occur, lowering levels of polymorphism (but for an exception see discussion in Cain, 1983). One would expect that freshwater populations, due to their seasonal nature, could again experience frequent bottlenecks, resulting in lower levels of polymorphism than marine populations where many species also have widely dispersed, planktonic larvae. Indeed, mean heterozygosity in freshwater populations was intermediate to terrestrial and marine values. In each of the habitats, outcrossers had the highest and facultative selfers or selfers the lowest heterozygosity, as would be expected. However, since many authors originally classified populations as selfers only because of low heterozygosity, these results could be somewhat circular. Freshwater parthenogens, interestingly, had the highest average heterozygosity. This suggests the existence of apomictic clones within these populations, as is also seen in parthenogenetically reproducing water fleas (Lynch, 1984) or brine shrimp (Browne *et al.*, 1984).

However, the interpretation of the literature survey was confounded by a number of gaps in the data available on genic polymorphism in gastropods. For example, is selfing a common reproductive mode in other habitats besides terrestrial ones? Although the ability to self might be advantageous in terrestrial habitats because of the patchy nature of the proper microenvironments and consequent low densities of conspecifics, we cannot be certain that predominately selfing populations also occur in either freshwater or marine

habitats, but have as of yet not been studied. Similarly, one wonders if parthenogens occur in other habitats besides freshwater. Vail (1978) reports that parthenogenesis is more frequent in upriver viviparid populations, where densities are low and chances of meeting males infrequent. If this is the advantage for parthenogens in freshwater, why have not parthenogens evolved (or more likely been studied) in terrestrial habitats since terrestrial snails are so patchily distributed? Finally, the available data are confounded by taxonomic bias. Most of the terrestrial snails are hermaphroditic pulmonates, capable of self-fertilization, whereas most of the aquatic and marine populations are dioecious prosobranchs.

Overall, this study points to the need for more work on levels of genetic variation in gastropods. We need further studies on the degree of genetic variation in polygenic traits like life histories both among and within populations. Contrasts between temporary and permanent ponds, as well as other important environmental parameters, would also be welcome. Russell-Hunter (1983) suggests the need for incorporation of factors like feeding niche (grazers vs. detritivores), reproductive modes (viviparity vs. oviviparity), possession of planktonic larvae, and colonization ability as well. We also need to fill in the gaps present in studies of allozyme variation, even though existing work is more thorough than studies on variation in polygenic traits. In particular, more work is needed on the degree of allozyme polymorphism among and within populations of freshwater pulmonates. Although we know much about variation in life histories and secondary production among populations of freshwater pulmonates (see reviews in Russell-Hunter, 1978; McMahon, 1983), much less is known of the underlying genetic variation for these traits among and within the same populations.

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Appendix 1. Terrestrial gastropods. Observed heterozygosity and mating system (? or mixed = facultative selfing).

SPECIES	H_o	MATING SYSTEM	STUDY
Milax sowerbyi (Férussac)	0.126	outcross	Foltz *et al.*, 1984
M. budapestensis (Hazay)	0.117	outcross	Foltz *et al.*, 1984
Limax maximus L.	0.027	outcross	Foltz *et al.*, 1984
L. pseudoflavus Evans	0.007	?	Foltz *et al.*, 1984
L. marginatus Müller	0.034	outcross	Foltz *et al.*, 1984
Deroceras caruanae (Pollonera)	0.049	outcross	Foltz *et al.*, 1984
D. reticulatum (Müller)	0.192	outcross	Foltz *et al.*, 1984
Milax gagates (Draparnaud)	0.013	outcross	Noble, unpubl.
Limax tenellus Müller	0.028	outcross	Noble, unpubl.
Deroceras agreste (L.)	0.0	selfer	Noble, unpubl.
Arion ater ater L.	0.0	mixed	Foltz *et al.*, 1982a
A. a. rufus L.	0.059	outcross	Foltz *et al.*, 1982a
A. lusitanicus Mabille	0.082	outcross	Foltz *et al.*, 1982a
A. subfuscus (A) (Draparnaud)	0.062	outcross	Foltz *et al.*, 1982a
A. subfuscus (B)	0.0	mixed	Foltz *et al.*, 1982a
A. circumscriptus Johnston	0.0	selfer	Foltz *et al.*, 1982a
A. silvaticus Lohmander	0.0	selfer	Foltz *et al.*, 1982a
A. hortensis Férussac	0.041	outcross	Foltz *et al.*, 1982a
A. intermedius Normand	0.0	selfer	Foltz *et al.*, 1982a
A. distinctus Mabille	0.186	outcross	Foltz *et al.*, 1982a
A. owenii Férussac	0.044	outcross	Foltz *et al.*, 1982a
Cerion bendalli Pilsbry and Vanatta	0.048	outcross	Woodruff, 1975
Deroceras laeve (Müller)	0.005	mixed	Foltz *et al.*, 1982b
Helix aspera (Müller)	0.200	?	Selander and Kaufman, 1975
Rumina decollata (L.)	0.0	selfer	Selander and Kaufman, 1975
Sphincterochila aharonii (Kobelt)	0.042	?	Nevo *et al.*, 1983
S. cariosa (Oliver)	0.043	outcross	Nevo *et al.*, 1983
S. fimbriata (Bourguignat)	0.104	outcross	Nevo *et al.*, 1983
S. prophetarum (Bourguignat)	0.074	?	Nevo *et al.*, 1983
S. zonata (Bourguignat)	0.079	?	Nevo *et al.*, 1983
Theba pisana (Müller)	0.105	?	Nevo *et al.*, 1981
Partula gibba Bruguiere	0.0	selfer	Johnson *et al.*, 1977
P. mirabilis Crampton	0.167	outcross	Johnson *et al.*, 1977
P. olympia Crampton	0.156	outcross	Johnson *et al.*, 1977
P. otaheitana Férussac	0.175	outcross	Johnson *et al.*, 1977
P. suturalis Pfeiffer	0.167	outcross	Johnson *et al.*, 1977
P. taeniata Mörch	0.134	outcross	Johnson *et al.*, 1977
Achatina fulica Bowdich	0.004	outcross	Selander and Ochman, 1983
Bradybaena similaris Férussac	0.083	outcross	Selander and Ochman, 1983
Cerion incanum (Burch and Kim)	0.051	outcross	Woodruff, 1978
Triodopsis albolabris (Say)	0.100	outcross	McCracken and Brussard, 1980
Xerocrassa seetzeni (Pfeiffer)	0.065	outcross	Nevo, 1978
Cepaea nemoralis (L.)	0.134	outcross	Jones *et al.*, 1980
C. hortensis (Müller)	0.117	outcross	Selander and Ochman, 1983
C. sylvatica (Draparnaud)	0.063	outcross	Selander and Ochman, 1983
Helix pomatia (L.)	0.030	outcross	Jarvinen *et al.*, 1976
Otala lactea Müller	0.196	outcross	Selander and Ochman, 1983
O. vermiculata Müller	0.117	outcross	Selander and Ochman, 1983
Oxychillas cellarius (Müller)	0.198	outcross	Selander and Ochman, 1983
Nymphophilus minckleyi Taylor	0.080	outcross	Selander and Ochman, 1983
Anguispira alternata (Say)	0.148	outcross	Selander and Ochman, 1983

Appendix 2. Freshwater gastropods. Observed heterozygosity and mating system.

SPECIES	H_o	MATING SYSTEM	STUDY
Goniobasis vanhyningiana Goodrich	0.031	outcross	Chambers, 1980
G. floridensis (Reeve)	0.077	outcross	Chambers, 1980
G. dickinsoni Clench and Turner	0.066	outcross	Chambers, 1980
G. athearni Clench and Turner	0.182	outcross	Chambers, 1980
G. albanyensis Lea	0.184	outcross	Chambers, 1980
G. curvicostata (Reeve)	0.078	outcross	Chambers, 1980
Melanoides tuberculata (Müller)	0.306	outcross	Livshits *et al.*, 1984
M. tuberculata (Müller)	0.111	parth	Livshits *et al.*, 1984
Campeloma decisa (Say)	0.095	parth	Selander *et al.*, 1978
C. decisa (Say)	0.033	parth	Selander *et al.*, 1978
Lymnaea elodes	0.088	mixed	This study (all populations)
Biomphilaria straminea (Dunker)	0.082	outcross	Woodruff *et al.*, 1985
B. glabrata (Say)	0.30	outcross	Woodruff *et al.*, 1985
B. havanensis (Pfeiffer)	0.091	outcross	Woodruff *et al.*, 1985
B. alexandria (Ehrenberg)	0.068	outcross	Woodruff *et al.*, 1985
Campeloma geniculum (Conrad)	0.250	parth	Karlin *et al.*, 1980
C. parthenum Vail	0.375	parth	Karlin *et al.*, 1980
Potamopyrgus jenkinsi Smith	0.138	parth	Selander and Ochman, 1983
Viviparous contectoides (Binney)	0.112	outcross	Selander and Ochman, 1983
Physa heterostropha (Say)	0.171	outcross	Selander and Ochman, 1983
Helisoma trivolvis Say	0.136	outcross	Selander and Ochman, 1983

Appendix 3. Marine gastropods. Observed heterozygosity and mating system.

SPECIES	H_o	MATING SYSTEM	STUDY
Adalaria proxima (Alder and Hancock)	0.082	outcross	Havenhand *et al.*, 1986
Onchidoris muricata (Müller)	0.059	outcross	Havenhand *et al.*, 1986
Thais haemastoma L.	0.106	outcross	Garton, 1984
T. lamellosa (Gmelin)	0.017	outcross	Garton and Stickle, 1985
Cerithium scabridum Philippi	0.168	?	Lavie and Nevo, 1986
C. rupestre (Risso)	0.035	?	Lavie and Nevo, 1986
Crepidula onyx Sowerby	0.161	outcross	Woodruff *et al.*, 1986
C. adunca Sowerby	0.052	outcross	Woodruff *et al.*, 1986
C. fornicata (L.)	0.045	outcross	Hoagland, 1985
Austrocochlea constricta Fisher	0.168	outcross	Mulley, 1981
Bathybembix bairdii (Dall)	0.162	outcross	Siebenaller, 1978
Cerithium scabridum	0.620	outcross	Ritte and Pashtan, 1982
C. caeruleum Sowerby	0.635	outcross	Ritte and Pashtan, 1982
Nassarius obsoletus (Say)	0.166	outcross	Gooch *et al.*, 1972
Littorina rudis (Dautzerberg and Fisher)	0.153	outcross	Ward and Warwick, 1980
L. arcana Ellis	0.132	outcross	Ward and Warwick, 1980